

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA85042 sequence which had the following nucleotide sequence

hybridization probe

5'-CCAGCCTTTGAATGGTACAAAGGAGAGAAGAAGCTCTTCAATGGCC-3' (SEQ ID NO:621)

RNA for construction of the cDNA libraries was isolated from human fetal brain tissue.

5 DNA sequencing of the clones isolated as described above gave the full-length DNA sequence for a full-length PRO4993 polypeptide (designated herein as DNA94832-2659 [Figure 229, SEQ ID NO:611]) and the derived protein sequence for that PRO4993 polypeptide.

10 The full length clone identified above contained a single open reading frame with an apparent translational initiation site at nucleotide positions 305-307 and a stop signal at nucleotide positions 1361-1363 (Figure 229, SEQ ID NO:611). The predicted polypeptide precursor is 352 amino acids long, has a calculated molecular weight of approximately 38,429 daltons and an estimated pI of approximately 6.84. Analysis of the full-length PRO4993 sequence shown in Figure 230 (SEQ ID NO:612) evidences the presence of a variety of important polypeptide domains as shown in Figure 230, wherein the locations given for those important polypeptide domains are approximate as described above. Clone DNA94832-2659 has been deposited with ATCC on June 15, 1999 and is assigned ATCC deposit no. 240-PTA.

15 An analysis of the Dayhoff database (version 35.45 SwissProt 35), using the ALIGN-2 sequence alignment analysis of the full-length sequence shown in Figure 230 (SEQ ID NO:612), evidenced sequence identity between the PRO4993 amino acid sequence and the following Dayhoff sequences: P_W05152; LAMP_HUMAN; P_W05157; P_W05155; I56551; OPCM_RAT; AMAL_DROME; DMU78177_1; I37246; and NCA1_HUMAN.

EXAMPLE 144: Isolation of cDNA Clones Encoding Human PRO1559, PRO725 and PRO739

25 A consensus sequence was obtained relative to a variety of EST sequences as described in Example 1 above. Based upon an observed homology between this consensus sequence and an EST sequence contained within Incyte EST clone No. 4242090, Incyte EST clone No. 4242090 was purchased and its insert was obtained and sequenced. It was discovered that the insert sequence encoded a full-length protein designated herein as PRO1559 (Figure 232; SEQ ID NO:614). The DNA sequence of the insert (DNA68886) is shown in Figure 231 (SEQ ID NO:613).

30 A cDNA sequence isolated in the amylase screen described in Example 2 above is herein designated DNA43301. The DNA43301 sequence was then compared to a variety of expressed sequence tag (EST) databases which included public EST databases (e.g., GenBank) and a proprietary EST DNA database (LIFESEQ™, Incyte Pharmaceuticals, Palo Alto, CA) to identify existing homologies. The homology search was performed using the computer program BLAST or BLAST2 (Altschul et al., Methods in Enzymology 266:460-480 (1996)). Those comparisons resulting in a BLAST score of 70 (or in some cases 90) or greater
35 that did not encode known proteins were clustered and assembled into consensus DNA sequences with the program "phrap" (Phil Green, University of Washington, Seattle, Washington). The consensus sequence obtained therefrom is herein designated DNA45458. Based on the DNA45458 consensus sequence, oligonucleotide probes were generated and used to screen a human fetal brain (LIB153) library prepared as

described in paragraph 1 of Example 2 above. The cloning vector was pRK5B (pRK5B is a precursor of pRK5D that does not contain the SfiI site; see, Holmes et al., *Science*, 253:1278-1280 (1991)), and the cDNA size cut was less than 2800 bp.

PCR primers (forward and reverse) were synthesized:

forward PCR primer (45458.f1) 5'-CCAAACTCACCCAGTGAGTGTGAGC-3' (SEQ ID NO:619)

5 reverse PCR primer (45458.r1) 5'-TGGGAAATCAGGAATGGTGTCTCC-3' (SEQ ID NO:620)

Additionally, a synthetic oligonucleotide hybridization probe was constructed from the consensus DNA45458 sequence which had the following nucleotide sequence

hybridization probe (45458.p1)

5'-CTTGTTTTCACCATTTGGGCTAACTTTGCTGCTAGGAGTTCAAGCCATGCC-3' (SEQ ID NO:621)

10 In order to screen several libraries for a source of a full-length clone, DNA from the libraries was screened by PCR amplification with the PCR primer pair identified above. A positive library was then used to isolate clones encoding the PRO725 gene using the probe oligonucleotide and one of the PCR primers.

15 A full length clone was identified that contained a single open reading frame with an apparent translational initiation site at nucleotide positions 161-163 and ending at the stop codon found at nucleotide positions 455-457 (Figure 233; SEQ ID NO:615). The predicted polypeptide precursor is 98 amino acids long, has a calculated molecular weight of approximately 11,081 daltons and an estimated pI of approximately 6.68. Analysis of the full-length PRO725 sequence shown in Figure 234 (SEQ ID NO:616) evidences the presence of the following: a signal peptide from about amino acid 1 to about amino acid 20, a potential N-glycosylation site from about amino acid 72 to about amino acid 75 and a tyrosine kinase phosphorylation site from about amino acid 63 to about amino acid 70. Clone DNA52758-1399 has been deposited with ATCC on April 14, 1998 and is assigned ATCC deposit no. 209773.

20 Analysis of the amino acid sequence of the full-length PRO725 polypeptide suggests that it possesses no significant sequence similarity to any known protein. However, an analysis of the Dayhoff database (version 35.45 SwissProt 35) evidenced some degree of homology between the PRO725 amino acid sequence and the following Dayhoff sequences, POL_BLVAU, PSSP_RAT, CELC36C5_7, AF019234_1, I48862, P_R12498, P_P10125, P_R26861, A64527 and P_W20495.

25 DNA52756, as shown in Figure 235 (SEQ ID NO:617) and which encodes native PRO739 polypeptide (Figure 236; SEQ ID NO:618) was obtained from GenBank.

30 EXAMPLE 145: Identification of Receptor/Ligand Interactions

In this assay, various PRO polypeptides are tested for ability to bind to a panel of potential receptor molecules for the purpose of identifying receptor/ligand interactions. The identification of a ligand for a known receptor, a receptor for a known ligand or a novel receptor/ligand pair is useful for a variety of indications including, for example, targeting bioactive molecules (linked to the ligand or receptor) to a cell known to express the receptor or ligand, use of the receptor or ligand as a reagent to detect the presence of the ligand or receptor in a composition suspected of containing the same, wherein the composition may comprise cells suspected of expressing the ligand or receptor, modulating the growth of or another biological or immunological activity of a cell known to express or respond to the receptor or ligand, modulating the immune response of cells or toward

cells that express the receptor or ligand, allowing the preparation of agonists, antagonists and/or antibodies directed against the receptor or ligand which will modulate the growth of or a biological or immunological activity of a cell expressing the receptor or ligand, and various other indications which will be readily apparent to the ordinarily skilled artisan.

The assay is performed as follows. A PRO polypeptide of the present invention suspected of being a ligand for a receptor is expressed as a fusion protein containing the Fc domain of human IgG (an immunoadhesin). Receptor-ligand binding is detected by allowing interaction of the immunoadhesin polypeptide with cells (e.g. Cos cells) expressing candidate PRO polypeptide receptors and visualization of bound immunoadhesin with fluorescent reagents directed toward the Fc fusion domain and examination by microscope. Cells expressing candidate receptors are produced by transient transfection, in parallel, of defined subsets of a library of cDNA expression vectors encoding PRO polypeptides that may function as receptor molecules. Cells are then incubated for 1 hour in the presence of the PRO polypeptide immunoadhesin being tested for possible receptor binding. The cells are then washed and fixed with paraformaldehyde. The cells are then incubated with fluorescent conjugated antibody directed against the Fc portion of the PRO polypeptide immunoadhesin (e.g. FITC conjugated goat anti-human-Fc antibody). The cells are then washed again and examined by microscope. A positive interaction is judged by the presence of fluorescent labeling of cells transfected with cDNA encoding a particular PRO polypeptide receptor or pool of receptors and an absence of similar fluorescent labeling of similarly prepared cells that have been transfected with other cDNA or pools of cDNA. If a defined pool of cDNA expression vectors is judged to be positive for interaction with a PRO polypeptide immunoadhesin, the individual cDNA species that comprise the pool are tested individually (the pool is "broken down") to determine the specific cDNA that encodes a receptor able to interact with the PRO polypeptide immunoadhesin.

In another embodiment of this assay, an epitope-tagged potential ligand PRO polypeptide (e.g. 8 histidine "His" tag) is allowed to interact with a panel of potential receptor PRO polypeptide molecules that have been expressed as fusions with the Fc domain of human IgG (immunoadhesins). Following a 1 hour co-incubation with the epitope tagged PRO polypeptide, the candidate receptors are each immunoprecipitated with protein A beads and the beads are washed. Potential ligand interaction is determined by western blot analysis of the immunoprecipitated complexes with antibody directed towards the epitope tag. An interaction is judged to occur if a band of the anticipated molecular weight of the epitope tagged protein is observed in the western blot analysis with a candidate receptor, but is not observed to occur with the other members of the panel of potential receptors.

Using these assays, the following receptor/ligand interactions have been herein identified: PRO337 binds to PRO4993, PRO1559 binds to PRO725, PRO1559 binds to PRO700 and PRO1559 binds to PRO739.

Deposit of Material

The following materials have been deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, USA (ATCC):

<u>Material</u>	<u>ATCC Dep. No.</u>	<u>Deposit Date</u>
DNA39987-1184	ATCC 209786	April 21, 1998
DNA40625-1189	ATCC 209788	April 21, 1998